European Journal of Biology and Medical Science Research

Vol.1, No.1, March 2013, pp.15-22

Published by European Centre for Research Training and Development UK (www.ea-journals.org)

EVALUATION OF HYPOGLYCEMIC PROPERTIES OF *MUCUNA COCHICHINENSIS* UNRIPE *CARICA PAPAYA* AND UNRIPE *MUSA PARADISIACA* FLOUR BLENDS

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Abstract: *Quantitative and qualitative phytochemical analysis of Mucuna Cochichinensis, unripe Carica papaya and unripe Musa paradisiaca flour blends were carried out. Alkaloid ranges from 1.20-2.20, tannin ranges from 0.16-2.4, saponin ranges from 1.19-1.67 and flavoniod ranges from 1.72-2.63%. MPPB had the highest phytochemical content. The hypoglycemic activity of the different flour blends where investigated on alloxan diabetic rats using Acc-check Active gluconometer daily for two weeks. Samples elicited hypoglycemic potential with sample MPPB giving the highest hypoglycemic activity reducing the hypoglycemic blood glucose concentration from 13.10mMol/l to 4.33mMol/l. The presence of high phytochemical in MPPB reversed hyperglycemia caused by alloxan- diabetics in male wister rats. MPPB is seen to be more effective and may be used for the treatment and management of diabetes mellitus*.

Key words: Hypoglycemic, Mucuna cochichimesis, Carica papaya Musa paradisiacal

1.0 Introduction

The therapeutic benefit of food have been known for a long time. In the 1900s the important role of diet in disease prevention and health promotion came to the forefront once again (Halser, 2002). Medicinal plants are now getting more attention than ever before because they have the potential of myriad benefit to the society or indeed to the entire mankind especially in the line of nutrition, medicine and pharmacology. The medicinal values of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Igwe et al., 2012). Some of the most important bioactive phytochemical constituent are alkaloids, flavonoids, tannins saponins, phenolic compound, essential oil and many more (Edeoga and Gomina 2000). Despite the great strides that have been made in the understanding and management of diabetes, the disease and the disease related complication are increasing unabated (Tiwari and Madhusudana 2002). Insulin therapy and oral hyperglycemic agents has shortcomings such as ineffectiveness in oral administration, short shelf life and requirement of constant refrigeration and in the event of excess dosage fatal hypoglycemia limit its usage (Anuradha et al., 2004).Therefore the search for more effective and safer hypoglycemic agents has continued to be an important area of investigation in the plant kingdom which culminated in the discovery of many herbal hypoglycemic agents and functional foods.

The hypoglycemic, antimicrobial anti-inflammatory, analgesic and antioxidant properties of plant extract have been reported (Adeyemi et al ., 2004)

Mucuna Cochichinesis is an annual leguminous climber. The major use of *Mucuna* at present is as green manure and cover crops for farmers. It has nutritional potential as a rich source of protein (Bressani 2002).*Carica Papaya* is cultivated for its fruit pawpaw. Pawpaw is favoured as breakfast and ingredients in jellies. The juice makes a popular beverage. Fruit extracts have pronounce bacteriocidal activities. Plantain(*Musa paradisiaca*) is a perennial plant with almost world wide distributed. It is low in fat, high in fibre and starch. It is rich in potassium and other minerals.This study therefore investigates phytochemical constituents present in *Mucuna Cochichinensis Musa paradisiaca* and *carica papaya* and the

possible hypoglycemic effects of the flour blends meal in alloxan diabetic rats which in turn may be used in the management and treatment of diabetes mellitus.

2.0 Materials and Methods

Mucuna cochichinensis, Carica papaya and *Musa paradisiaca* were purchased from Ahiaohuru market in Aba.

Mucuna Cochichinesis were sorted, weighed, washed, boiled at 100^0c for 90 minutes, dehulled, dried at 65^0c to a constant weight, milled and sieved into flour.

Musa paradisiaca were washed, weighed, peeled, sliced, blanched for 4 minutes, dried at 70^0c to a constant weight, milled and sieved into flour.

Carica papaya were washed, weighed, peeled, sliced, blanched for 4 minutes, dried at 70^0c to a constant weight, milled and sieved into flour.

6 different experimental diet samples were formulated with a total of 100g per diet.

Composition of the experimental diet on dry weight basis g/100g

Ingredient	samples					
	MPPA	MPPB	MPPC	MPPD	MPPE	Nutrend (Control)
Mucuna	40	50	30	50	40	-
Musa paradisiaca	40	30	50	40	40	-
Carica papaya	20	20	20	10	10	-

Phytochemical Determination

The method of Harbone (1973), AOAC (1984) were used to determine qualitatively and quantitatively the presence of flavonoid, alkaloid, tannin and saponin.

Hypoglycemic activities of the blended flour:

Thirty five albino wister rats of about 10-15 weeks old and each weighing about 200g were purchased form department of Biochemistry FUTO and housed in a screened wired guaze cages in the animal house of the department of Biology Abia State Polytechnic Aba. The animals were acclimatized for two weeks with provision of commercially formulated feed. (Growers, pellet, Grand Nigeria feed) and water. Their cages were properly swept, washed with clean water and disinfectant (Izal and Dettol) for proper health of the animals and ventilation throughout the study period. The animals after acclimatization for the days were fasted overnight but allowed free assess to water before the experiment..

This was done in order to ascertain the normal glucose concentrations of the individual rats before induction of diabetes.

The animals were divided into seven different experimental groups. 1-7. Group 1 served as normal control. Group 2 serves as diabetic control. Group 3,4,5,6 and 7 were induced diabetes with alloxan 90mg/kg body weight and fed with the different diets.

The blood glucose level of group 1 - 7 (animal) were checked daily for 14 days using Accu Check Active Gluconometer (Roche Switzerland). The glucose concentration more than 140g/dl was considered diabetic.

The blood glucose levels of the animals were checked daily for 14 days to ascertain either a rise or fall in the glucose levels to know the effect of the diet on the blood glucose level of the rats.

Treatment Groups

1. Non diabetic fed with normal feed rat/nutrend
2. Induce diabetes with alloxan 90mg/kg body weight and fed with normal feed rat/nutrend
3. Induce diabetes with alloxan 90mg/kg body weight and fed with sample MPPA
4. Induce diabetes with alloxan 90mg/kg body weight and fed with sample MPPB
5. Induce diabetes with alloxan 90mg/kg body weight fed with sample MPPC
6. Induce diabetes with alloxan 90mg/kg body weight fed with sample MPPD
7. Induced diabetes with alloxan 90mg/kg body weight fed with sample MPPE

3.0 Results and Discussion

Table 1 **Qualitative analysis of the phytochemical of *Mucuna Cochichinesis, Carica Papaya* and *Musa Paradisiaca* flour blends.**

Phytochemicals	MPPA	MPPB	MPPC	MPPD	MPPE	Nutrend
Alkaloid						
a. Drogendorffs test	+	+	+	+	+	-
b.Mayers Test	+	+	+	+	+	-
Flavoniods						
a. Dilute ammonia test	+	+	+	+	+	-
b.Shinodas test	+	+	+	+	+	-
Tannins						
a. Folin Denis reagent	+	+	+	+	+	-
FecL(111) solution	+	+	+	+	+	-
Saponins						
a. Frothing test	+	+	+	+	+	-
b. Emulsion test	+	+	+	+	+	-

Qualitative phytochemical screening of the different flour blends indicated the presence of alkaloids, flavonoids, saponins and tannins as shown in Table 1.

Table 2 **Quantitative phytochemical analysis**

Sample	Alkaloid	Tannins	Saponins	Flavonoid
MPPA	1.74^{b}	0.24^{a}	1.31^{b}	2.50^{a}

MPPB.	2.20[a]	0.27[a]	1.7[a]	2.63[a]
MPPC	0.97[b]	0.23[a]	1.67[a]	1.93[b]
MPPD	1.69[b]	0.18[b]	1.40[b]	1.83[b]
MPPE	1.20[d]	0.16[b]	1.15[b]	1.72[b]
LSD	0.02	0.03	0.01	0.02

Means on the same row with the same superscript are not significantly different ($p<0.05$)

Alkaloids: The values ranged from 1.20 to 2.20%. (Table 2). There were significant differences in the alkaloid content with sample MPPB having the highest alkaloid content. Alkaloids are used for medication as recreational drug, local anesthetics, stimulants, an analgesic and as anti malarial drug. Alkaloids can also be lethal if used in the wrong quantities (Harbones et al., 1995).

Flavonoids: The values ranged from 1.72 to 2.63%. (Table 2). There were significant differences ($p<0.05$) in the flavonoid content. Sample MPPB has the highest flavonoid content. Flavonoids have antioxidant properties and help to neutralize free radical damages. By neutralizing over reactive oxygen- containing molecules they prevent cell damages . Flavonoid, enhance the power of vitamin C and prevent excessive inflammation in the body also have anti viral and anti bacterial activity (Silagy, 1994). To sustain healthy tissues and achieve the correct balance of hormone and antioxidants in the body, flavonoids are recommended by some nutritionist.(Brown et al., 1998). Flavonoids have also been implicated as antoxidants both in physiological and diseased states. For instance tea flavonoids have been reported to reduce the oxidation of low density lipoprotein, lower the blood level of cholesterol and triglycerides (Erdman, 2007).

Saponins: There were significant differences in the saponin content ($p<0.05$). Values ranges from 1.15 to 1.7 (Table 2). Saponins are bile acid sequestrants that have the ability to bind with cholesterol and pathogens entering the body forming molecules too large to be absorbed through the intestinal wall. Thus cholesterol and pathogen molecule are then carried through the digestive system forcing them to be eliminated. This help to relieved, stress from bodies vital immune system. Clinical reports have also shown that the immune system is affected by saponins in ways that lower cholesterol levels and help to protect the human body against cancers (Pathirana et al., 1980). This hypocholesterolemic effect has been ascribed to a complexation of saponin with cholesterol and bile acids, which results in a decrease in their absorption from the intestines (Matsuura, 2001). Saponins also reduce blood lipids (Chunmei et al., 2006), lower the risk of developing cancer (Messina and Bennike, 1989) and lower blood-glucose response (Sajadi Tabassi et al., 2007). This statement implies that the consumption of MPPB will have the above effect in the body.

Tannins: There were no significant differences in the tannin content ($p<0.05$). Values ranged from 0.16 to 0.24 (Table 2). According to Liu et al.,(2004) tannins have shown potential antiviral properties ,antibacterial properties and antiparasitic potential. According to Enujiugha and Agbade (2000) tannins usually forms insoluble complexes with proteins thereby interfering with bioavailability. Poor palatibility is generally attributed on high tannin diet (Mehansha et al., 1987). Tannin are capable of lowering available protein by antagonistic competition and can therefore elicit protein deficiency syndrome "Kwashiorkor". Results are well below value that are toxic.

TABLE 3 **Blood glucose concentration of normal control, diabetic control and MPP flour blends.**

	1	2	3	4	5	6	7
Time	Normal Control	Diabetic Control	MPPA	MPPB	MPPC	MPPD	MPPE
Days	mmol/l	mmol/l	mmol/l	mmol/l	mmol/l	mmol/l	mmol/l
1.	3,25[a]	12.80[b]	12.90[b]	13.10[c]	12.04[a]	12.95[b]	13.15[c]
2.	3.67[a]	12.90[b]	11.40[c]	11.80[c]	11.81[c]	11.90[c]	12.30[b]
3.	4.10[a]	13.10[b]	9.90[c]	9.40[c]	11.40[c]	10.50[c]	10.80[c]
4.	4.20[a]	13.50[b]	9.10[c]	8.50[c]	10.50[c]	9.80[c]	9.40[c]
5.	5.23[a]	13.90[b]	8.20[c]	7.10[d]	9.60[c]	9.40[c]	9.00[c]
6.	4.13[a]	14.0[b]	8.10[c]	5.00[d]	9.00[c]	8.20[c]	8.40[c]
7	4.10[a]	14.14[b]	7.60[c]	4.00[c]	8.00[c]	8.01[c]	8.01[c]
8.	4.04 [a]	14.67[b]	6.80[c]	3.53d	7.90c	7.80c	7.90c
9	3.80[a]	16.00[b]	6.00[c]	3.40[d]	6.00[c]	6.80[c]	6.80[c]
10	3.97 [a]	18.90[b]	5.51[c]	3.80[d]	5.50[c]	5.40[c]	5.50[c]
11.	4.04[a]	20.20[b]	5.40[c]	4.10[d]	5.00[c]	4.15[c]	4.70[c]
12.	4.10 [a]	25.80[b]	4.80[a]	3.50[a]	5.20[a]	4.20[a]	4.40[a]
13.	3.90[a]	-----	4.32[a]	3.80[a]	5.30[a]	4.31[a]	4.20[a]
14.	4.13[a]	------	4.13[a]	3.73[a]	4.90[a]	4.50[a]	4.30[a]

The result on the blood glucose concentration of normal control, diabetic control and diabetic rats fed with the different samples for 14 days are shown in Table3.

For the group 1 the variations in the glucose level were observed to fall within normal 3.8mMol/l – 7.8mMol/l. For group 2 which is the diabetic control the glucose level, were observed to increase to a level that killed all the rats before the 14 days. There were gradual reduction of the glucose level in group 3 rats until the glucose level came to normal after the ninth day, for group 4 rats there were reduction of glucose level to the normal range after the 7 day and persisted in that level. Other groups were observed slow reduction of the glucose to normal range after the tenth day.

Alloxan is a specific toxic substance that destroy the B- cells providing a state of primary deficiency of insulin without affecting other islet types (Goldener and Gomori 1964) hence alloxan was selected to induce diabetes in the present study. Toxicity of alloxan is elicited through its reduction by glutathione to dialuric acid in which redox cycling process generates reactive oxygen species (ROS), that damages the beta cell (Malaisse, 1982). It is known that diabetes mellitus and its complication can arise due to oxidative stress.

Oxidative stress is initiated by free radical which seek stability through electron pairing with biological macro molecules such as proteins lipids and DNA in healthy cells an cause protein and DNA damage along with lipid peroxidation (Hazra, 2008).

All living cells protect themselves against free radical damage by enzymes such as super oxide dismutase (SOD) catalase (CAT) or compounds such as ascorbic acid (vitamin C) tocopherol

(vitamin E) glutathione (GSH). In diabetes mellitus these protective mechanism are disrupted. Many synthetic drugs protect against oxidative damage but they have adverse side effects. An alternative solution to the problem is to consume natural anti-oxidants from food supplement and traditional medicine.

The MPPB flour blend significantly ($P< 0.05$) reduced blood glucose concentration in Alloxan-induced diabetic wister rats. This suggest that the flour formulation maybe acting on the pancreas with possible enhancement of insulin production and its release. Alloxan monohydrate induces chemical diabetic in a wide variety of animal species by damaging the insulin secreting pancreatic B-cells, resulting in decreased endogenous insulin release, which will in turn lead to decreased utilization of glucose by the tissues (Omamoto et al 1981). The flour formulation significantly lowered the glucose levels of the rats and also successfully countered the Alloxan-induces hyperglycemia. This thus confirms the hypoglycemic activities and possible insulin tropic activities of the formulations.

Feeding of the rats with MPPB produced significant ($P<0.05$) decrease in blood glucose concentration in Alloxan – induced diabetic rats. The five flour blends produced hypoglycemic activity which did not however vary much from each other. The flour blends (table 2) are hypoglycemic and have beneficial health outcomes with regards to hyperglycemia a major biochemical marker of diabetes mellitus.

In Table 2, flavonoid and alkaloid were highest in sample MPPB and since flavonoid are anti-oxidants, this could be a contributive factor for the notable reduction in glucose level of the diabetic rat. The diabetic control all died because of absence of phytochemical in their meal. Currently available drugs for treatment of diabetes mellitus have a number of limitations such as adverse effects and high rate of secondary failure (Koski 2004). As there is a growing trend towards using natural remedies as adjuncts to conventional therapy traditional used plants food provide a useful source of new hyperglycemic compounds. The present study demonstrated hypoglycemic properties of *Mucuna Cochichinensis, unripe Carica, papaya* and unripe *Musa paradisiaca* flour blends meal.

4.0 Conclusion

The presence of abundant metabolites (phyto-chemical) in the constituent flour blends used in our studies has been established qualitatively and quantitatively. The plants *Mucuna cochichinensis,* unripe *Carica papaya* and *Musa paradisiaca* were found to be richer in flavonoid than other phytochemical quantified.

The presence of high concentration of these phytochemical as established by our analysis maybe responsible in part for the efficacy of the flour formulations used in this study. This justifies the use of these flour in ethno-medicinal practice in the treatment/management of diabetes mellitus.

The flours exhibited very strong hypoglycemic activities. Steady reduction of fasting plasma glucose level by the various combinations throughout the period of study scientifically confirms the use of flour in glycemic control in ethno-medicine. The hypoglycemic potentials of these plants and their combination as observed in this research give a very strong scientific support for the use of these flours in the preparation of the anti diabetic formula. We have scientifically justified the use of MPPB flour blend in the treatment and management of diabetes by ascertaining that the formulation MPPB have the efficacious potentials

* hypoglycemic potential
* Antioxidant potential,
* Non toxic, no side effect, no mortality.

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